Substituted Pyrazolopyridines as Potent and Selective PDE5 Inhibitors: Potential Agents for Treatment of Erectile Dysfunction

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Received January 18, 2001

Introduction. Erectile dysfunction (ED) was largely an unmet medical need prior to the introduction of sildenafil [Viagra (**1**), Chart 1] in 1998.[1] Sildenafil is a potent inhibitor of phosphodiesterase type 5 (PDE5),[2] and it was originally studied for the treatment of angina before its effectiveness in treating ED was serendipitously discovered. Despite its success, sildenafil has several notable side effects such as headache, nausea, cutaneous flushing, and visual disturbances.[2a,3] These side effects may be attributed to the limited selectivity of **1** against other PDE isozymes, most notably PDE1 and PDE6 (Table 1). Thus, the need exists for improved PDE5 inhibitors possessing greater PDE isozyme selectivity and demonstrating fewer side effects. In this Letter, we detail a new series of potent PDE5 inhibitors represented by **5**, which have a much improved PDE isozyme selectivity profile compared to **1**.

PDE5 is a member of the phosphodiesterase family of enzymes which are responsible for the hydrolysis of cGMP and/or cAMP.[3] PDE5 is the primary cGMP hydrolyzing enzyme present in the *corpus cavernosum*, the tissue in the penis which is engorged with blood during an erection. Upon sexual stimulation, nitric oxide (NO) is released from nonadrenergic, noncholinergic neurons in the penis. NO activates guanylyl cyclase, which in turn produces cGMP. cGMP initiates a protein phosphorylation cascade, which causes a decrease in intracellular calcium within *corpus cavernosal* smooth muscle cells, resulting in vasorelaxation, inflow of arterial blood, and an erection.[4] Inhibition of PDE5 increases the effective concentration of cGMP in the *corpus cavernosum*, enhancing the above-described effects.

The therapeutic benefit of sildenafil in ED results from its potent inhibition of PDE5. However, it is only modestly selective toward other PDE isoforms, notably PDE1 and PDE6 (Table 1). At relevant therapeutic doses of sildenafil, it is likely that measurable inhibition of PDE1 and PDE6 occurs. This may be the cause of some of the side effects noted with sildenafil use. Consistent with this hypothesis is the fact that the incidence of side effects is dose-related. The visual disturbances associated with sildenafil treatment can be linked to inhibition of PDE6, the sole cGMP PDE in the retina. Other side effects may be due, at least in part, to nonspecific inhibition of PDE1, which is abundant throughout most of the vasculature. Thus, more selective PDE5 inhibitors should be of substantial clinical interest.[5] This communication describes the discovery of a novel series of pyrazolopyridine derivatives which are potent, selective, efficacious, and orally bioavailable PDE5 inhibitors.

Results and Discussion. Compound **2** (Chart 1, PDE5 IC_{50} = 1 nM) was reported as a potent PDE5 inhibitor by Kumar and Dority from Sanofi-Winthrop.[6] Using **2** as a template for a substructure search, we chose a series of pyrazolopyridines for PDE5 screening. This effort identified **4** (Chart 1, PDE5 IC_{50} = 180 nM) as a nonselective PDE5 inhibitor with modest potency. Exploration of the SAR of **4** was then initiated using parallel synthesis (Scheme 1). By varying the amine substituent located at C4 of the pyrazolopyridine heterocycle, we identified 3-chloro-4-methoxyphenylmethylamine (**10**) as the optimal amine for PDE5 inhibitory potency. Watanabe and co-workers have made a similar observation in a series of phthalazines (e.g., **3**, Chart 1).[7] A second round of parallel synthesis was undertaken to examine the role of the amide at C5 (Scheme 1). Using **6** as the template, a large library of amides was synthesized (Scheme 1). This effort afforded many potent and selective PDE5 inhibitors, as exemplified by **5**, with PDE5 IC_{50}'s < 10 nM. The best PDE5 selectivity profiles were found in those amides which contained hydrogen-bonding functionalities, such as pyridines (**5**), phenols, ethers (**7**), esters, alcohols (**8**), and amides (**9**) (Chart 1).[10]

Among the compounds in the above-described amide library, compound **5** distinguished itself based on physical properties (such as solubility) and in vitro biological properties (Table 1).[8] The potency and selectivity of **5** were much improved compared to sildenafil (**1**). Most notable was the significantly improved selectivity for PDE5 versus PDE6 and PDE1.

The ability of a compound to potentiate relaxation of *corpus cavernosal* tissue strips in vitro has been used as a functional measure of the PDE5 inhibition.[9] This model requires that the drug penetrates *corpus cavernosal* cells, since PDE5 is an intracellular enzyme. When evaluated in rabbit *corpus cavernosal* tissue strips, **5** demonstrated equal efficacy compared to sildenafil (**1**, Table 2). Thus, in addition to being a more potent and selective PDE5 inhibitor in vitro (compared with sildenafil), compound 5 was at least equivalent to sildenafil in its functional PDE5 activity in rabbit *corpus cavernosal* tissue.

Examination of the pharmacokinetic profile of **5** in rats (21 μmol/kg) and dogs (23 μmol/kg) demonstrated equal or better exposure of drug in rats and dogs compared with **1** (Table 2). The relatively short terminal half-life of 5 in both species was well-suited for the on-

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10.1021/jm0155042 CCC: $20.00
Published on Web 03/06/2001

Chart 1. PDE5 Inhibitors

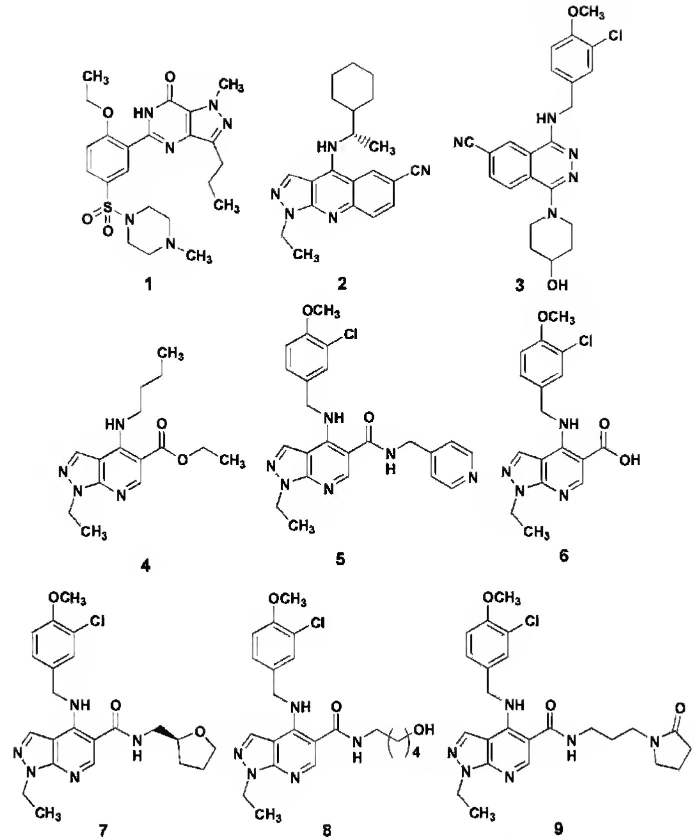


Table 1. PDE5 Inhibition and Isozyme Selectivities[8]

		IC$_{50}$ ratio				
compd	IC$_{50}$ PDE5 (nM)	1/5	2/5	3/5	4/5	6/5
1[a]	1.6 ± 0.6	160	12000	4100	2900	7 ± 3.2
2[b]	1.0	NA	NA	NA	NA	NA
3[c]	0.6	45000	1600	29000	1900	NA
4[a]	180 ± 80	43	45	42	1	2
5[a]	0.8 ± 0.5	6400	9200	3800	600	47 ± 17
6[a]	38	NA	NA	NA	NA	NA
7[a]	1.8 ± 0.3	2500	8000	5800	760	22 ± 15
8[a]	1.4 ± 0.6	2700	5200	2400	340	35 ± 7.5
9[a]	1.6 ± 0.7	2900	3000	4100	460	19 ± 16

[a] Determined in house; enzyme sources: PDE1, bovine heart; PDE2, rat kidney; PDE3, human platelet; PDE4, rat kidney; PDE5, human platelet; PDE6, bovine retina. All IC$_{50}$ determinations are averages based on 3 determinations. [b] Taken from ref 5. [c] Taken from ref 7. NA: not available.

Table 2. Characteristics of 5 Compared to Sildenafil (1)[a]

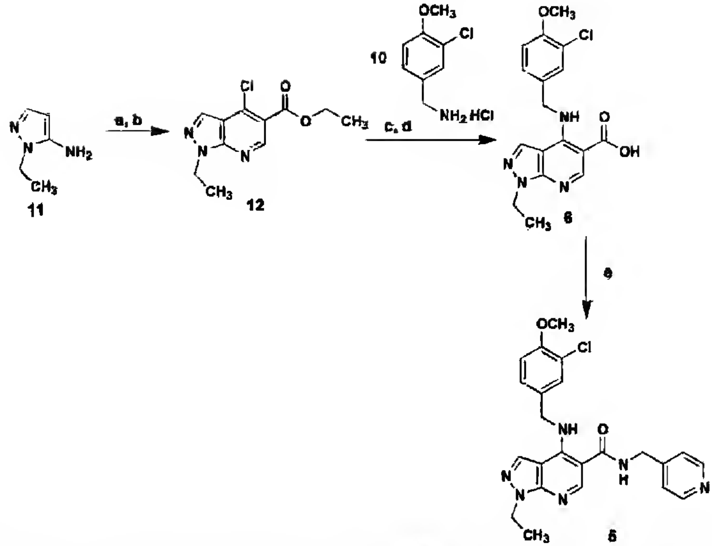
compd	% F in male rats (R) or dogs (D)	half-life (h) in male rats (R) or dogs (D)	EC$_{50}$ in vitro strip assay (nM)
1 (sildenafil)	15 (R) 54 (D)	0.3 (R) 5.2 (D)	42 ± 8
5	33 (R) 66 (D)	1.2 (R) 2.3 (D)	44 ± 3

[a] Oral bioavailability (% F) and half-life of sildenafil taken from the Viagra NDA.

demand dosing consistent with medication for this quality-of-life disorder (Table 2). The safety profile of **5** in rats was evaluated at doses of 10, 50, and 250 mg/kg, and there were no drug-related effects at 10 and 50 mg/kg. The only adverse effect observed at 250 mg/kg was limited to lower body weight in male rats (6% lower than control). There were no drug-related gross-pathologic lesions observed at any dose in both male and female rats. Thus, **5** was found to be orally bioavailable in rats and dogs with no apparent safety issues.

In summary, using two rounds of parallel synthesis, we have identified a series of potent PDE5 inhibitors based on a pyrazolopyridine template (i.e., **4**) obtained via directed screening.[10] This series of compound demonstrated potent PDE5 inhibition in vitro. Compound **5** distinguished itself by its selectivity profile. Its PDE1 and PDE6 isozyme selectivities were superior to that of **1** (sildenafil), albeit with decreased PDE4 selectivity.

Scheme 1[a]



[a] Reagents: (a) neat (EtO)CH=C(CO_2Et)$_2$ (1.1 equiv), 120 °C, 5 h; (b) neat $POCl_3$ (3.0 equiv), 110 °C, 2 h, 50% from **11**; (c) 3-Cl-4-MeO-benzylamine·HCl (**10**; 1.05 equiv), Et_3N (3.0 equiv), EtOH, 80 °C, 3 h; (d) NaOH (2.0 equiv), EtOH/H_2O, reflux, 2 h, 87% from **12**; (e) EDAC·HCl (1.2 equiv), HOBT (1.2 equiv), 4-Py-CH_2NH_2 (1.1 equiv), Et_3N (5.0 equiv), rt, 12 h, 90%.

Compound **5** also showed comparable in vitro functional PDE5 inhibition when compared with **1**. Additional studies demonstrated that **5** had a good pharmacokinetic profile in two species with no safety concerns in rats.[10] Thus, because of its improved PDE isozyme selectivity profile compared with sildenafil, compound **5** might be expected to have fewer PDE-related side effects if used for the treatment of ED.

Acknowledgment. We thank Drs. David Rotella, Yingzhi Bi, and Jacques Roberge for their helpful discussions throughout this project. We thank Dr. Jack Gougoutas, Mr. John DiMarco, and Ms. Mary Malley for the X-ray structure determination.

Supporting Information Available: Experimental details, X-ray structure of **5**, and PDE assay protocol. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Enzyme inhibition assays were carried out as described in: Rotella, D.; Sun, Z.; Zhu, Y.; Krupinski, J.; Pongrac, R.; Seliger, L.; Normandin, D.; Macor, J. E. N-3-Substituted imidazoquinazolinones: potent and selective PDE5 inhibitors as potential agents for treatment of erectile dysfunction. *J. Med. Chem.* **2000**, *43*, 1257–1263.

(9) A rabbit *corpus cavernosal* tissue strip assay was used to evaluate the functional effects of the PDE5 inhibitors on smooth muscle relaxation. Electrical field stimulation causes the release of NO from endogenous nerve terminals, resulting in a frequency-dependent relaxation of phenylephrine-precontracted strips of rabbit *corpus cavernosum* even in the absence of a PDE5 inhibitor. Addition of a PDE5 inhibitor potentiates the electrical field stimulation relaxation. This potentiation is expressed as an increase in the relaxation integral at 32 Hz, with the maximum relaxation integral in the presence of vehicle alone defined as 100%. Thus, a value greater than 100% indicates a potentiation effect. Sildenafil was used as a positive control in each experiment. Both compounds were tested at concentrations of 3, 10, 30, 100, 300, and 1000 nM in separate strips from at least 4 rabbits. The ability of the compounds to induce direct relaxation of the phenylephrine-precontracted *corpus cavernosal* strips was also evaluated.

(10) A more comprehensive examination of the SAR within this series will be presented separately.

JM0155042